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MASTERS IN MEDICINE (MICROBIOLOGY) RESEARCH REPORT

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CONTENTS **PAGE**

1.1 TITLE PAGE i
1.2 DECLARATION ii
1.3 ABSTRACT iii
1.4 ACKNOWLEDGEMENTS iv
1.5 LIST OF FIGURES v
1.6 LIST OF TABLES vi
1.7 SUBMISSIBLE ARTICLE vii

INTRODUCTION 1
METHODS 2
Study design 2
Definitions 2
Sample size 3
Bacterial culture and identification 3
Antimicrobial susceptibility testing 3
Data Analysis 4
RESULTS 4
Distribution of isolated uropathogens from CMJAH 4
Fosfomycin susceptibility testing results 4
Multidrug Resistance (MDR) rates 8
Fosfomycin susceptibility compared with other oral agents used to treat *Enterobacteriaceae* causing UTIs 9
DISCUSSION 11
LIMITATIONS 16
CONCLUSION 17
REFERENCE 18
1.8 APPENDIX 23
Ethics Clearance Letter

1.1 TITLE PAGE

FOSFOMYCIN SUSCEPTIBILITY OF UROPATHOGENS AT CHARLOTTE MAXEKE JOHANNESBURG ACADEMIC HOSPITAL (CMJAH)

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1.2 DECLARATION BY STUDENT AND CO-AUTHORS REGARDING THE SUBMISSIBLE ARTICLE

I, Dr. Lesego Moetsana Mothibi, student number 589881, declare that this research report (in submissible article format) is my original work and that I contributed significantly towards the research findings included in this report.

Signature of Student..... Date: 31 October 2018

.....

Name of Supervisor.....Dr. Trusha Nana.....

Signature of Supervisor .....

Date ...31 October 2018.....

Name of Supervisor.....Dr. Norma Bosman.....

Signature of Supervisor .....

Date .....31 October 2018.....

Agreement by co-authors:

By signing this declaration, we, the co-authors, Dr. Norma Bosman and Dr. Trusha Nana agree that Dr. Lesego Mothibi contributed significantly to the submissible article. She developed the protocol for the study, collected the data, analyzed it and did the write up of the article. We assisted her by supervising the project.

We agree for her to use the article as her research report towards acquiring the Masters in Medicine degree.

Signature of co-author .....

Date..... 31 October 2018

Signature of co-author.....

Date..... 31 October 2018

1.3 ABSTRACT

Fosfomycin Susceptibility of Uropathogens at Charlotte Maxeke Johannesburg Academic Hospital (CMJAH)

Abstract

Background:

Multidrug resistant urinary pathogens are becoming widespread both in community and hospital setting. Safe yet effective treatments are a priority. Fosfomycin is an antibacterial that displays good activity against most bacteria causing urinary tract infections (UTIs), including multidrug-resistant (MDR) bacteria.

Objectives:

The aim of this study was to evaluate the susceptibility of fosfomycin for uropathogens isolated from a microbiology laboratory in a South African tertiary teaching hospital.

Methods:

We conducted a retrospective analysis of laboratory reports for uropathogens isolated at Charlotte Maxeke Johannesburg Academic Hospital (CMJAH) from September 2015 to August 2017. Antimicrobial susceptibility testing of the isolates was performed using the Kirby-Bauer disk diffusion method or the Vitek® 2 system (bioMérieux SA, France) according to the Clinical and Laboratory Standards Institute (CLSI).

Results:

Overall susceptibility of fosfomycin for the *Enterobacteriaceae* was 95.7%; 95% confidence interval (CI) 95.1- 96.2. The predominant causative organisms isolated were *Escherichia coli* (*E. coli*), *Klebsiella pneumoniae* and *Enterococcus faecalis.* Fosfomycin activity against *E. coli* was 98.1%. Other members of the *Enterobacteriaceae* family in this study were also highly susceptible to fosfomycin except for the *Morganella* species. The overall susceptibility for fosfomycin against the gram positives was 98.6%. There were 37.9% multidrug resistant *Enterobacteriaceae* (MDRE) isolated during the study period. Fosfomycin remained highly

active against MDRE, 94.4% for extended-spectrum β-lactamase (ESBL) producers and 90.7% for carbapenem-resistant *Enterobacteriaceae* (CRE). None of the Methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant Enterococcus (VRE) isolates tested fosfomycin resistant. The overall in vitro susceptibility was significantly higher for fosfomycin (p-value = 0.0001) compared to amoxicillin/clavulanic acid, cephalexin, cefuroxime, ciprofloxacin, trimethoprim/sulfamethoxazole, and nitrofurantoin.

Conclusion:

This study confirmed the very high susceptibility of fosfomycin against urinary tract infection pathogens isolated at our institution. The data adds to the growing evidence that fosfomycin may be a good option for therapy of UTIs in the era of increasing antimicrobial resistance.

1.4 ACKNOWLEDGEMENTS

I would like to sincerely thank Dr. Trusha Nana & Dr. Norma Bosman for their time and expertise in assisting me with this project.

I would also like to extend my gratitude to the staff members at Charlotte Maxeke Johannesburg Academic hospital microbiology laboratory, NHLS, for their support with this project.

1.5 LIST OF FIGURES

Figure 1: Susceptibility of fosfomycin against *Enterobacteriaceae* 7

Figure 2: Susceptibility of fosfomycin against gram-positive bacteria 7

Figure 3: Multidrug- Resistant *Enterobacteriaceae* isolated at high risk units for MDRO 9

Figure 4 Susceptibility of fosfomycin compared with other antibiotics used to treat *Enterobacteriaceae* causing UTIs 10

1.6 LIST OF TABLES

Table 1: Distribution of isolated uropathogens from the hospital 6

Table 2: Percentage of multidrug resistance *Enterobacteriaceae* isolates 8

1.7 SUBMISSIBLE ARTICLE

Introduction

Urinary tract infections (UTIs) have become the most common bacterial infections, equally found in the community and in hospitals. [1] Antibiotics such as ciprofloxacin, trimethoprim/sulfamethoxazole, and nitrofurantoin were used successfully as therapy for UTIs. [2] However, due to the increased drug resistance to these antibiotics, the choice of empiric antimicrobials for UTIs has become more challenging. [3, 4] As a result, there is a growing need to find novel treatment options or to re-examine existing agents. There is growing interest in old antimicrobials such as fosfomycin.

Fosfomycin is a broad-spectrum antimicrobial agent that inhibits the synthesis of the bacterial cell wall of both gram-negative and gram-positive bacteria. [5] The Infectious Diseases Society of America's (IDSA) 2010 guidelines on the treatment of uncomplicated UTIs recommend fosfomycin 3-g oral single-dose as one of the first-line treatment options. [6] Fosfomycin has a pharmacokinetic profile that favours its use for treatment of lower UTIs; it is rapidly absorbed after oral administration, the mean peak urinary concentration takes place within 4 hours, while concentrations adequate to suppress most uropathogens are sustained for 24 to 72 hours. [2, 5] Fosfomycin displays good activity against most bacteria-causing UTIs, including multidrug-resistant (MDR) organisms. [5] It is well tolerated with minimal side effects, such as diarrhoea and headache, and is not contraindicated in pregnancy. [5] Clinical trials have shown that a single 3g dose of fosfomycin is as effective as a 7- to a 10-day regimen using other oral antibiotics to treat acute uncomplicated cystitis. [5] Literature suggests that this agent may be a suitable antibiotic to treat lower UTIs in most regions, for both hospitalised and ambulatory patients. [6]

The oral formulation of fosfomycin has only recently become available at our centre. Susceptibility testing for fosfomycin on urinary isolates has since been introduced at Charlotte Maxeke Johannesburg Academic Hospital (CMJAH) microbiology laboratory. Consequently, there was a lack of information regarding the susceptibility rates of fosfomycin among uropathogens isolated at our centre. In this study, we aimed to analyse retrospective data for fosfomycin susceptibility of gram-negative and gram-positive bacteria isolated from urine samples and to compare its activity

with that of other oral antimicrobials usually used to treat lower UTIs. Furthermore, the burden of MDR uropathogens was also established. This information will assist in determining the role of oral fosfomycin at our centre. In addition, it will help with the formulation of guidelines for therapy of lower UTIs locally.

Methods

Study design

This is a retrospective laboratory-based study undertaken at CMJAH microbiology laboratory. CMJAH is a 1088-bed university hospital. The hospital offers secondary, tertiary and quaternary services, as well as outpatient clinics. It also serves as a referral centre for several hospitals.

Antimicrobial susceptibility testing (AST) data for uropathogens isolated at CMJAH from September 2015 to August 2017 were used for analysis. Furthermore, the burden of MDR uropathogens for specific high-risk units for multidrug-resistant organisms (MDROs) was established. The data for this study were retrieved from the National Health Laboratory Service (NHLS) Corporate Data Warehouse (CDW). The study was accepted by the Human Research Ethics Committee (Medical) of the University of the Witwatersrand, approval number M170935.

Definitions

Multidrug resistance was defined as acquired non-susceptibility to at least one agent in three or more antimicrobial classes. [7]

AmpC- β-lactamase producers (Amp C): Organisms resistant to cefoxitin were considered to be Amp C producers.

Extended-spectrum β-lactamases (ESBLs): Organisms resistant to ceftriaxone or ceftazidime were considered to be ESBL producers.

Carbapenem-resistant *Enterobacteriaceae* (CRE): Organisms resistant to ertapenem were considered to be carbapenem resistant.

Sample size

In total 8906 bacterial isolates were included in the study. Repeated tests viz, bacteria cultured from the same patient, were considered to be duplicate organisms representing a single infection if isolated within 2 weeks and were removed from the analysis. Fosfomycin results were not available for all the *Enterobacteriaceae* in the data retrieved from the CDW. In addition, fosfomycin susceptibility testing of gram-positive bacteria was performed on 76 selected non-duplicate isolates identified in the microbiology laboratory between June 2017 and August 2017.

Bacterial culture and identification

The microbiological diagnosis of a UTI requires $\geq 10^5$colony-forming units (CFU/ mL) of a single microorganism in a midstream collected urine, or $\geq 10^4$ CFU/ mL of a single microorganism in catheter samples, or any bacterial growth in urine obtained by suprapubic route. [8] Any urine sample that grew more than two microorganisms was considered contaminated. Bacterial culture and identification of urine specimens were performed according to the CMJAH Microbiology Laboratory Urine Standard Operating Procedures (SOP). Urine specimens were inoculated on 5% blood agar and MacConkey agar plates and incubated overnight at 35-37° C. The bacterial species identification was performed using the API® 20E kit (bioMérieux SA, France) or the Vitek® 2 system (bioMérieux SA, France).

Antimicrobial susceptibility testing

Routine AST of the isolates was performed using the Kirby-Bauer disk diffusion method or the Vitek® 2 system (bioMérieux SA, France) according to the Clinical and Laboratory Standards Institute (CLSI). [9-11] Fosfomycin susceptibility was determined by the Kirby-Bauer disk diffusion method. The plates were incubated aerobically at 35°C - 37°C for 18 - 24 hours. The results were interpreted according to the CLSI guidelines breakpoints for the corresponding year. [9-11] The CLSI provides fosfomycin interpretive breakpoints for *Escherichia coli* (*E.coli*) and *Enterococcus faecalis* (*E.faecalis*) only. The fosfomycin interpretive breakpoints for *E. coli* were used other for gram-negative bacteria, whilst the interpretive breakpoints for *E. faecalis* were

used to interpret fosfomycin susceptibility of all the gram-positive bacteria. [2] For *Enterobacteriaceae*, CLSI interpretative breakpoints for cefazolin were used to predict susceptibility to cephalexin. Two bacterial strains were used for quality control (QC) of AST: *E. coli* (American Type Culture Collection (ATCC) 25922) and *Staphylococcus aureus* (*S. aureus*) (ATCC 25923). [9-11] Quality control was performed weekly.

Data Analysis

Fosfomycin and other oral antibiotic susceptibility results were reported as percentage-susceptible. The difference between the percentage of isolates susceptible to fosfomycin and other oral antibiotics was calculated using 95% Confidence Intervals (CI) and p-values.

The 95% CI was calculated using the Poisson distribution. A p-value < 0.05 was considered statically significant. All statistical analyses were conducted using STATA, version 14 (StataCorp, USA).

Results

Distribution of isolated uropathogens from CMJAH

The distribution of isolated uropathogens from CMJAH is shown in Table 1. From September 2015 to August 2017, a total of 9083 (21.9%) urines were culture positive. There were 7395 (81.4%) gram-negative bacilli, 1511 (16.6%) gram-positive cocci and 177 (2%) *Candida* species isolated. The predominant causative organisms were *E. coli*, *K. pneumoniae* and *E. faecalis*.

Fosfomycin susceptibility testing results

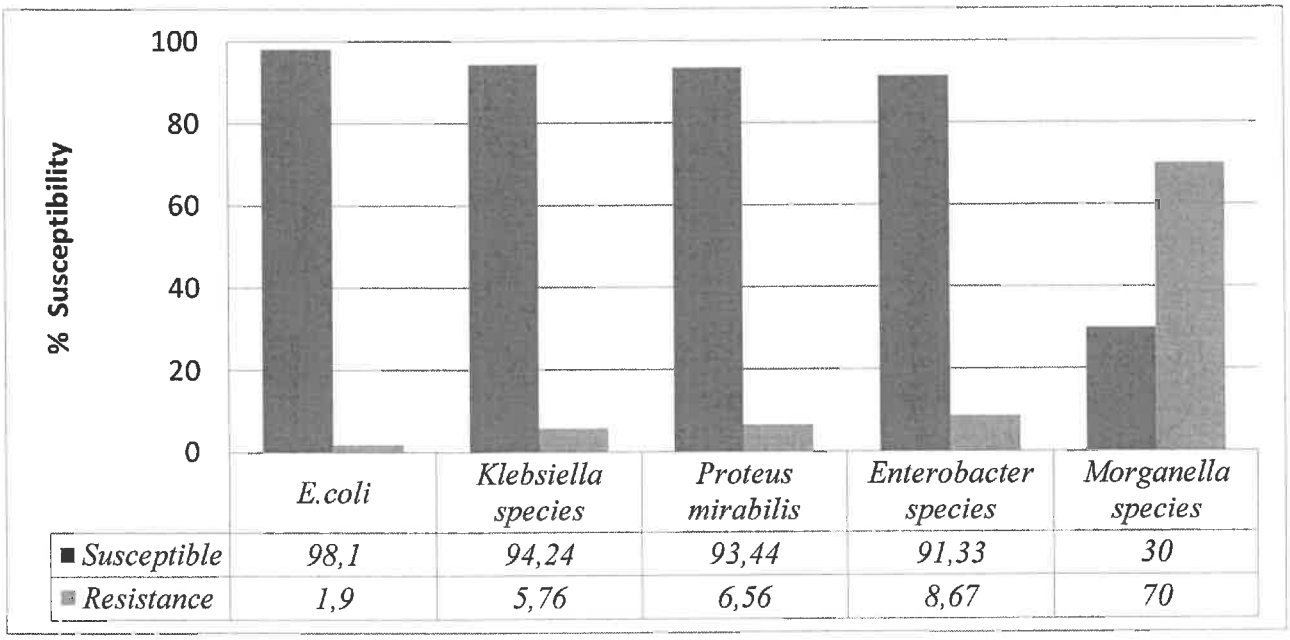
Fosfomycin susceptibility testing results were available for 4700 *Enterobacteriaceae*. The overall susceptibility of fosfomycin for the *Enterobacteriaceae* was 95.7%; 95% CI 95.1- 96.2; p-value of 0.0001. Fosfomycin activity against *E. coli* was 98.1%. Other members of the *Enterobacteriaceae* family in this current study also showed susceptibility to fosfomycin except for *Morganella* species (Figure 1).

A breakdown of the 76 gram-positive organisms tested for fosfomycin susceptibility demonstrated 47 (61.8%) *E. faecalis*, 15 (19.7%) *E. faecium*, 7 (9.2%) *S. aureus*, 4 (5.3%) *Streptococcus agalactiae* (*S. agalactiae*) and 3 (3.9 %) *Staphylococcus saprophyticus* isolates. Since *S. saprophyticus* is intrinsically resistant to fosfomycin, these isolates were removed from the analysis. The overall susceptibility for fosfomycin for the gram-positives was 98.6%. All the *S. aureus* isolates tested were susceptible to fosfomycin. Fosfomycin activity against enterococcal species, including the vancomycin-resistant enterococci (VRE) (n =3) strains, was excellent. A single *E. faecalis* isolate was resistant to fosfomycin (Figure 2).

Table 1. Distribution of isolated uropathogens from the hospital

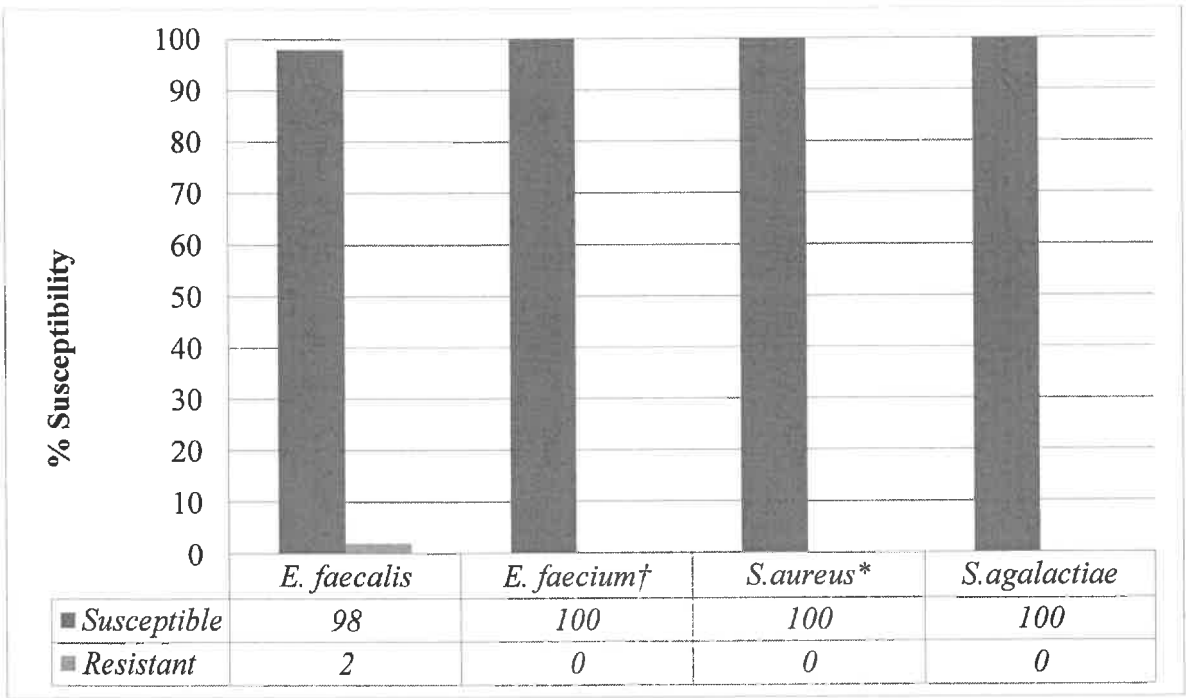
	N (%)
Total no. of specimens*	41 446
Total no. of specimens with uropathogens	9083
Total gram-negative bacilli isolated	7 395 (81.4)
E. coli	4 142 (56)
Klebsiella pneumoniae	1 252 (16.9)
Klebsiella oxytoca	98 (1.3)
Proteus mirabilis	475 (6.4)
Enterobacter species	314 (4.2)
Citrobacter species	129 (1.7)
Pseudomonas aeruginosa	364 (4.9)
Acinetobacter baumannii	241 (3.2)
Others	380 (5.1)
Total gram-positive cocci isolated	1 511 (16.6)
S. aureus	143 (9.5)
S. saprophyticus	12 (0.8)
E. faecalis	792 (52.4)
E. faecium	182 (12.1)
S. agalactiae	227 (15)
Others	155 (10.3)
Total *Candida* species	177 (2)
Candida albicans	97 (54.8)
Non-*albicans Candida* species	80 (45.2)

* Urine samples from September 2015 to August 2017 (study period)



	E.coli	*Klebsiella species*	*Proteus mirabilis*	*Enterobacter species*	*Morganella species*
Susceptible	*98,1*	*94,24*	*93,44*	*91,33*	*30*
Resistance	*1,9*	*5,76*	*6,56*	*8,67*	*70*

Figure 1. Susceptibility of fosfomycin against *Enterobacteriaceae*



	E. faecalis	*E. faecium†*	*S.aureus**	*S.agalactiae*
Susceptible	*98*	*100*	*100*	*100*
Resistant	*2*	*0*	*0*	*0*

Figure 2. Susceptibility of fosfomycin against gram-positive bacteria

†Includes VRE

*Includes MRSA.

Multidrug Resistance (MDR) rates

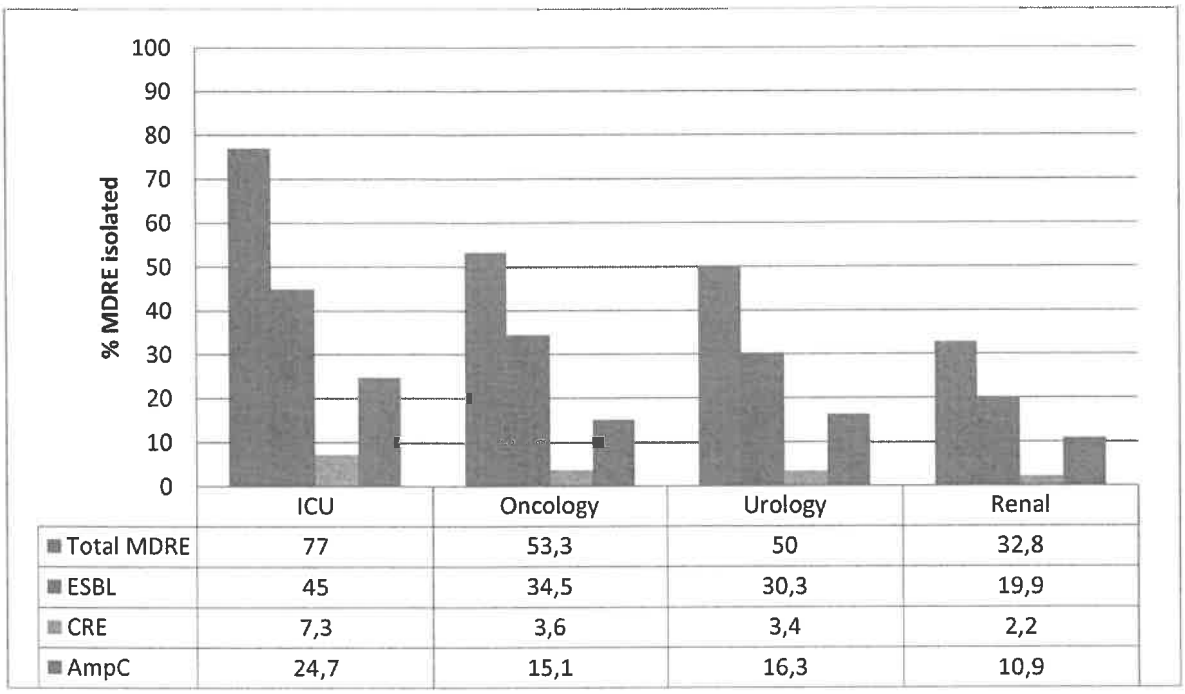
There were 2520 (37.9 %) multidrug-resistant *Enterobacteriaceae* (MDRE) isolated during the study period. The most common resistance mechanism was in ESBL producers. ESBL production was identified in 23.2% (n=1545) of the *Enterobacteriaceae* (Table 2). The majority of ESBL producers were *K. pneumoniae* 636 (41.2%) and *E. coli* = 623 (40.3%) (data not shown). Overall, 24 (17%) of *S. aureus* isolates were MRSA and 11 (1.1%) of the *Enterococcus* species isolates were VRE (data not shown). The majority of MDRE were isolated from patients admitted to ICUs (Figure 3).

For the subset of MDRE with fosfomycin results available, the fosfomycin susceptibility was: 94.4% for ESBL producers and 90.7% for carbapenem-resistant *Enterobacteriaceae* (CRE). All the MRSA and VRE isolates tested showed 100% susceptibility to fosfomycin (data not shown).

Table 2: Percentage of multidrug resistance *Enterobacteriaceae* isolates

MDRE	N (%)
Total number of *Enterobacteriaceae*	6652
Total number of MDRE	2520 (37.9%)
ESBL	1545 (23.2%)
AmpC	815 (12.3 %)
CRE	160 (2.4%)

MDRE = Multidrug-Resistant *Enterobacteriaceae*: AMP C= AmpC β-lactamase producers; ESBL = Extended spectrum β-lactamase producers; CRE= Carbapenem resistant *Enterobacteriaceae*



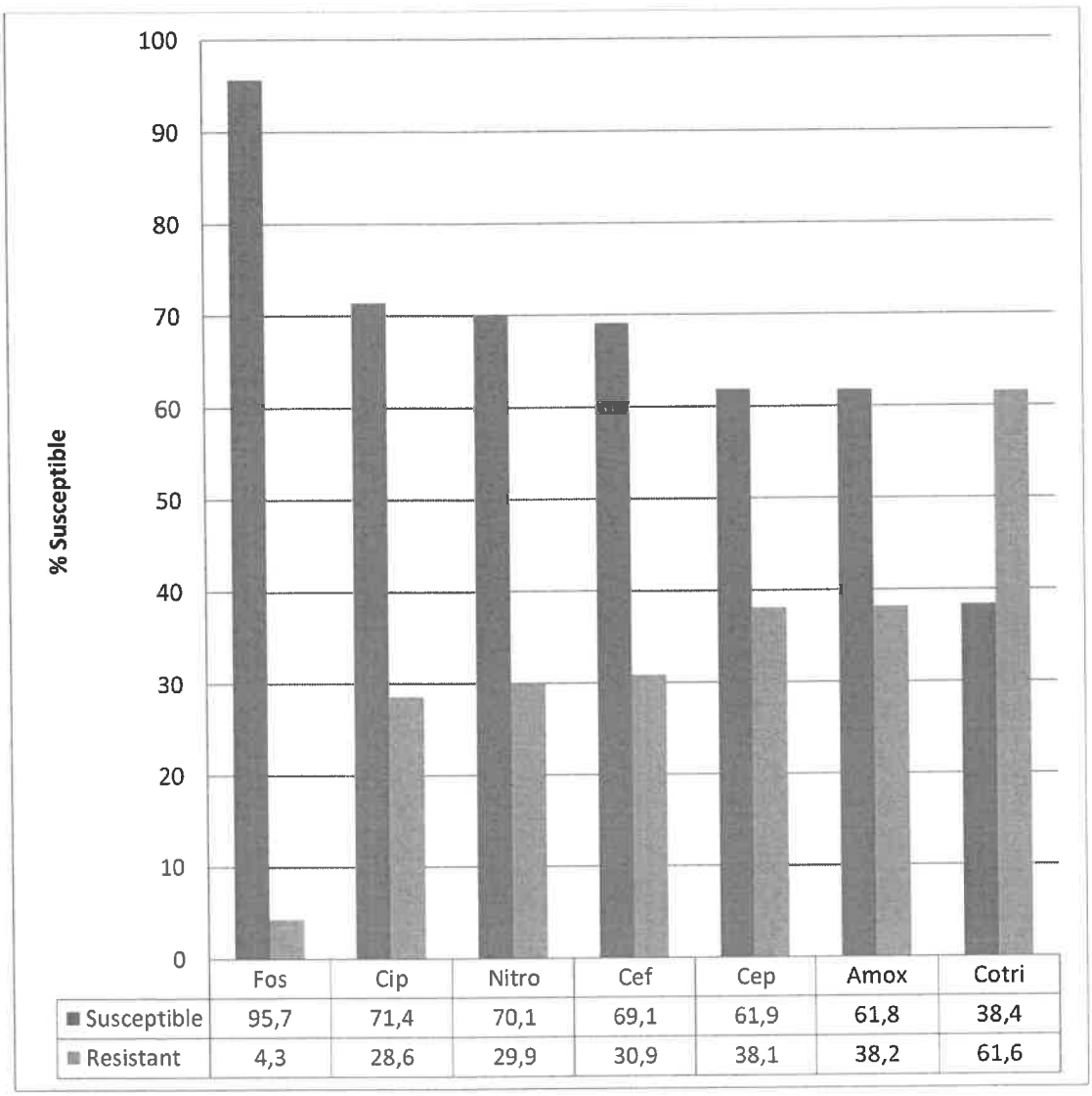
	ICU	Oncology	Urology	Renal
Total MDRE	77	53,3	50	32,8
ESBL	45	34,5	30,3	19,9
CRE	7,3	3,6	3,4	2,2
AmpC	24,7	15,1	16,3	10,9

Figure 3. Multidrug- Resistant *Enterobacteriaceae* isolated at high risk units for MDRO

MDRO = Multidrug-resistant organisms; ICU = Intensive care unit; AMP C= AmpC β-lactamase producers; ESBL = Extended spectrum β-lactamase producers; CRE= Carbapenem resistant *Enterobacteriaceae*

Fosfomycin susceptibility compared with other oral agents used to treat *Enterobacteriaceae* causing UTIs

In the present study, fosfomycin showed the highest susceptibility (95.7%, 95% CI: 95.1 -96.2) against the tested *Enterobacteriaceae*. There was a statistically significant difference between the susceptibility rates of fosfomycin (p= 0.0001) and all the other oral agents tested. Ciprofloxacin, cefuroxime and nitrofurantoin susceptibility rates were modest; however, they were much lower compared to that of fosfomycin. The lowest susceptibility was seen with trimethoprim/sulfamethoxazole (38.35%, 95% CI: 37.2 – 39.5) (Figure 4).



	Fos	Cip	Nitro	Cef	Cep	Amox	Cotri
Susceptible	95,7	71,4	70,1	69,1	61,9	61,8	38,4
Resistant	4,3	28,6	29,9	30,9	38,1	38,2	61,6

Figure 4. Susceptibility of fosfomycin compared with other antibiotics used to treat *Enterobacteriaceae* causing UTIs

UTIs = Urinary tract infections; Fos = fosfomycin; Cip = Ciprofloxacin; Nitro= Nitrofurantoin; Cef = Cefuroxime; Cep =Cephalexin; Amox = Amoxicillin-clavulanate; Cotri = Trimethoprim/sulphamethoxazole

Discussion

The key finding of this study is that fosfomycin shows outstanding activity against clinical uropathogens, including MDR isolates. Overall, the susceptibility of fosfomycin against *Enterobacteriaceae* was 95.7%. Fosfomycin activity against *E. coli* was higher than that of other *Enterobacteriaceae.* Significant susceptibility rates to fosfomycin were also found for *Klebsiella pneumoniae*, *Proteus mirabilis,* and the tested gram-positive isolates. These findings are similar to those reported in the literature. [4, 12-13] *Morganella* species strains displayed significantly reduced susceptibility compared to other *Enterobacteriaceae* (70% resistance rates). This is in keeping with other studies, where fosfomycin resistance rates for *Morganella morganii* range between 75% and 100%. [14-15] *M. morganii* is regarded as being intrinsically resistant to fosfomycin. [16]

The overall susceptibility of fosfomycin for gram-positives in this study was 98.6%. The most frequent gram-positive uropathogens were *Enterococcus* species, which were highly susceptible (98%) to fosfomycin. Research by Sultan *et al.* found that fosfomycin was highly effective against *Enterococcus* species and *Staphylococcus* species including VRE and MRSA. [17] Additional studies have shown that fosfomycin enhances the susceptibility of *S. aureus* to phagocytosis and has significant intraphagocytic bactericidal activity. [18] In addition fosfomycin is active against *S. aureus* biofilm production. [18] Bacterial biofilm can play an important role in recurrent UTIs and resistance to antimicrobial agents.

Fosfomycin also demonstrated good activity against UTIs caused by MDRO. In this current study, the susceptibility of fosfomycin for MDRE causing UTIs was 90.7% (CRE), 92.3% (AmpC), and 94.4% (ESBL). Comparable to this study, a systematic review of *E. coli* ESBL susceptibility to fosfomycin revealed susceptibility rates of more than 81%. [19]. All MRSA and VRE tested in this research were susceptible to fosfomycin. Due to the high fosfomycin susceptibility rates against uropathogens in our setting, fosfomycin is an appropriate choice of treatment for outpatients and hospitalised patients with suspected or culture-confirmed MDR (MDRE, VRE and MRSA) UTIs. A significant pharmacological benefit of fosfomycin is that it can be administered orally. The use of fosfomycin could prevent admission for treatment of MDR UTIs or decrease the length of hospital stay by allowing substitution of oral for intravenous therapy. Fosfomycin could also prove useful for the treatment of UTIs in circumstances where therapy with conventional antibiotics was

unsuccessful or is contraindicated. Furthermore, the use of aminoglycosides may be retained for MDR *Acinetobacter baumannii,* and *Pseudomonas aeruginosa* treatment.

Studies have demonstrated the clinical efficacy of fosfomycin as therapy for lower UTIs caused by both susceptible and MDR uropathogens. A report by Neuner confirmed microbiological cure rates in 71% of patients with UTIs caused by VRE and ESBL-producers. The study population included patients with co-morbidities such as diabetes mellitus, solid-organ transplant recipients and chronic kidney disease. [13] Another study by Nagel *et al.* compared clinical outcomes of fosfomycin in the hospital setting for patients with susceptible and MDR UTIs. A cure rate of 95% was documented. The majority of patients had UTIs caused by VRE (45%). [20] A cohort study of hospitalised patients treated with oral fosfomycin for uncomplicated or complicated UTIs showed favourable clinical outcomes, with cure rates of 75 to 90% for the different cohorts studied. Their study included patients with comorbidities and an equal proportion of community-acquired and nosocomial infections in the cohorts studied. [21]

Globally, the susceptibility rates of fosfomycin have continued to be quite stable since its introduction. Studies performed in three European countries, where parenteral and oral fosfomycin has been used since the 1970s, showed no significant decrease in fosfomycin susceptibility. [22] Fosfomycin exhibits a low level of cross-resistance to other antimicrobial agents because of its unique mechanism of action. [12] Furthermore, the administration of oral fosfomycin demonstrates no effect on the resistance characteristics of faecal flora.

Enterobacteriaceae are the common aetiological agents of UTIs. [3, 4] Our epidemiology is in keeping with the existing literature, where the majority of bacterial isolates were gram-negative bacilli (81.4%). The predominant causative organisms in this current study were *E. coli, K. pneumoniae* and *E. faecalis*. Even though gram-negative bacteria are the main causative organisms for complicated and uncomplicated UTIs, gram-positive bacteria are also important uropathogens. [3, 4] *S. saprophyticus, E. faecalis, Streptococcus agalactiae* are the common cause of community-acquired UTIs in pregnant women and the elderly. *E. faecalis* and *E. faecium* are the third leading cause of nosocomial UTIs. [23] The ratio of *E. faecalis* to *E. faecium* in this current study was 4: 1. The incidence of UTIs caused by *E. faecalis* has escalated gradually and now outnumbers *E. faecium* UTIs. [23]

Rates of both nosocomial and community-acquired UTIs caused by MDR bacteria are escalating. [3, 5, 24] In this study, 37.9% of *Enterobacteriaceae* were MDR. The ESBL phenotype resistance pattern was the common MDR pattern detected. β-lactamase producers can express resistance to other classes of antibiotics used to treat UTIs. [25] The increasing rate of resistance to multiple antibiotic classes and the high rate of Multidrug-Resistant *Enterobacteriaceae* (MDRE) may lead clinicians to use more carbapenems and colistin. Colistin is a potentially nephrotoxic antibiotic and is associated with neurological toxicity. [26] Carbapenems and colistin are parenteral antibiotics and necessitate patient hospitalisation, which is linked to substantial financial burdens. Systemic exposure to broad-spectrum antibiotics is also associated with increased selection of MDR pathogens. [27] This study shows that MRSA (17%) isolates are predominant uropathogens in our institution (data not shown). A comparable MRSA rate (14%) was reported in a study by Rajuduraipandi. [28] In contrast, higher rates of 80.9 % in Indore and 50.4% in eastern Uttar Pradesh were reported. [29 -30] This variability could be because of the difference in study design, population, antibiotic *prescribing habits* and geographic distribution.

This study did not look at the risk factors associated with MDRE. Rather, we focused on establishing the burden of MDRE uropathogens in selected wards. Previous studies have acknowledged that prior fluoroquinolone use within 3 months, a hospital stay for at least 48 hours, comorbidities, an indwelling urinary catheter, and urological procedures within the past 3 months are independent risk factors linked with MDRE UTIs. [31] The distribution of MDRE UTIs in the selected high-risk wards is shown in figure 3. The majority of MDRE UTIs were from patients admitted to ICU (77%). Indwelling urinary catheters, the length of ICU admission and selective pressure from the use of antimicrobial agents are the most likely risk factors for the high rates of MDRE in ICU. UTIs caused by ESBL producers were more common than other phenotypes and ranged between 45% and 19.9%. The findings emphasise the significance of antibiotic resistance surveillance data and the knowledge of the risk factors associated with MDR UTIs in guiding empiric therapy choices.

The initiation of appropriate antimicrobial agents empirically before urine culture results are available is of utmost importance. However, in order to optimize empiric therapy, local patterns

of antimicrobial resistance should be known. Antibiotic resistance rates and patterns differ by patient population type and change over time. [32] The IDSA 2010 guidelines recommend a 10% to 20% resistance threshold for the treatment of community-acquired UTIs using a particular antibiotic; above this threshold, the agent should not be used for empiric therapy. [6]

In our setting, when compared with other oral agents used for UTIs, isolates were more frequently susceptible to fosfomycin. Fosfomycin susceptibility rates for *Enterobacteriaceae* were the highest, followed by ciprofloxacin, nitrofurantoin and cefuroxime. Our data confirmed decreased ciprofloxacin susceptibility amongst the *Enterobacteriaceae* tested. Although fluoroquinolones are highly efficacious in a 3-day regimen, there has been a dramatic increase in resistance to these agents in gram-negative uropathogens in the past decade. A multicentre study conducted between 2007 and 2011 detected a significant decrease in ciprofloxacin susceptibility amongst *E. coli* causing UTIs in both the public and the private sector. Ciprofloxacin susceptibility in the public sector declined by 5% and by 7% in the private sector. The data in their study comprised of urine specimens from both the in-and outpatient settings. [24] Ongoing indiscriminate and widespread use of ciprofloxacin to treat different infections is expected to cause a further decline in the susceptibility of pathogenic microorganisms to ciprofloxacin. This is particularly concerning, as both the South African Antibiotic Stewardship Programme (SAASP) Adult Antibiotic Guidelines and the Standard Treatment Guidelines and Essential Medicines List (EDL) for South Africa (SA) recommend ciprofloxacin for management of cystitis and pyelonephritis in patients that are not severely ill and are able to tolerate oral medication. [33 - 34] Empirical treatment with ciprofloxacin would be inadequate in almost 30% of UTI cases. Therefore, the use of ciprofloxacin at our centre should be reserved for treatment of UTIs caused by bacteria with known susceptibility to this drug. A follow-up multicentre study should be undertaken to assess ciprofloxacin susceptibility rates.

Nitrofurantoin susceptibility (70.1%) was lower compared to findings from previous studies in our region, where the reported susceptibility was above 90%. [4, 17] Nitrofurantoin is an alternative antibiotic for the treatment of UTIs caused by MDRE, but it is contraindicated in advanced pregnancy and in patients with significant renal dysfunction. [35] Nitrofurantoin has a decent efficacy and a low predisposition to cause collateral damage. [4, 24]

Even though the first-generation cephalosporins reach high concentrations in the urine, they have high resistance rates when compared with other agents. [36] A study by Chen *et al.* found a significant decline over a ten-year period for the sensitivity of *E. coli* to first-generation cephalosporins. The sensitivity rate was 72% in 2003 and 28% in 2012. [37] In 2011, CLSI modified cefazolin breakpoints and this likely contributed to the higher observed resistance rates in this current study. Therefore, this antibiotic cannot be used for empiric UTIs treatment in our institution. The susceptibility of cefuroxime in our study was lower (61.9%) compared to that found by Lewis. [4] However our study did not distinguish between community-acquired and nosocomial UTIs. Previous widespread use of cefuroxime for other indications may have contributed to the declined susceptibility. Cefuroxime is currently not available in the state sector because it is not part of the Department of Health (DOH) essential drug list (EDL). [34]

Although trimethoprim/sulfamethoxazole is still widely used and recommended for acute cystitis therapy, the limitation is that local resistance rates of uropathogens should not exceed 20%, or the infecting strain should be confirmed to be susceptible. [6] In the present study, the rate of trimethoprim/sulfamethoxazole susceptibility (61.6%) was the lowest amongst the oral antimicrobials tested. A study conducted in South Africa's Gauteng province, similarly found trimethoprim/sulfamethoxazole to be the least efficacious antimicrobial agent against uropathogens causing community-acquired UTIs. [4] Trimethoprim/sulphamethoxazole has been used as a prophylactic agent against opportunistic infections in HIV/AIDS patients in SA. This may be contributing to the particularly high rates of resistance.

Fosfomycin has also been used off-label with success for complicated UTIs excluding pyelonephritis, perinephric abscess or bacteraemic UTIs. [38] The doses that have been used for complicated UTIs and MDRE vary in literature, for example, 3g every 48 -72 hours for 6 to 21 days has been used. Three doses are generally adequate, but the total length of therapy is guided by the resolution of the patient's symptoms. [38] Studies suggest that dose adjustment is not required in susceptible groups, such as pregnant women, patients of advanced age, and those with hepatic or renal failure. Therefore, it is a safe drug for patients with hepatic or renal dysfunction or for

those receiving concomitant nephrotoxic drugs. [5, 39] Fosfomycin may be used in children; however, the availability of pharmacokinetic data for neonates is very scanty. The dose that has been used for children less than 5 years is 1- 2 g, but this dose strength is currently not available in SA. [34]

Gardiner *et al.* found that the concentration attained by oral fosfomycin in the prostate is enough to prevent prostatic infection in patients undergoing transrectal ultrasound-guided biopsy. Fosfomycin may also be considered as an alternative treatment for prostatic infections caused by MDRE. [40] The usual dosage regimen is 3 g of oral fosfomycin 3 hours before and 24 hours after traumatic interventions and surgical procedures. It is given as a single dose for diagnostic procedures. [41] Studies that compared the efficacy of ciprofloxacin versus fosfomycin when used for prophylaxis of urological procedures demonstrated that fosfomycin was associated with lower rates of subsequent UTIs. [42-43]

Research suggest that surgical antibiotic prophylaxis is beneficial for preventing UTIs post urologic surgeries and procedures. [41] The selection of the antibiotic used for surgical prophylaxis must also be based on the local epidemiology of antibiotic resistance. [41] This study did not assess the role of fosfomycin in surgical prophylaxis for urological surgeries and procedures. However, based on the susceptibility of the microorganisms causing UTIs as shown in our research, fosfomycin could be used for surgical prophylaxis in our setting. It is vital to perform local studies to determine the role of fosfomycin above current antibiotic regimens used for surgical prophylaxis.

Limitations

This study has several limitations. It was a retrospective single centre study; therefore, the findings may not be generalised to other institutions with different susceptibility patterns and patient populations. As CMJAH is a referral hospital, antibiotic resistance rates may be higher than those found in local community hospitals. Confirmatory tests for AmpC- β -lactamase & ESBL production was not performed. Ertapenem was used as a surrogate marker of CRE, molecular confirmation of carbapenemase production was not performed. CLSI guidelines (M100-S25) recommended broth microdilution (BMD) as the reference method for the determination of colistin minimum inhibitory concentration (MICs). [9] Colistin broth microdilution was not performed in

this study; therefore, we were not able to comment on the rate of colistin resistance. The small sample size of gram-positives may limit the ability to make reliable conclusions about the activity of fosfomycin against these uropathogens in our setting. This needs to be addressed by performing a larger evaluation. This study was not designed to differentiate between community-acquired and nosocomial UTIs. Lastly, the study was not designed to assess clinical and microbiological outcomes.

Conclusion

This study is important in that it represents the first local comprehensive evaluation of fosfomycin against gram negative and gram positive uropathogens. Fosfomycin has been shown to have good activity against both susceptible and MDR uropathogens. These findings together with its favourable pharmacokinetic/ and side-effect profile, position it as an effective and safe alternative oral agent for the treatment of UTIs for hospitalised and ambulatory patients, particularly at our centre.

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1.8 APPENDIX

R14/49 Dr Lesego Mothibi

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)

CLEARANCE CERTIFICATE NO. M170935

NAME: (Principal Investigator)	Dr Lesego Mothibi
DEPARTMENT:	Clinical Microbiology and Infectious Diseases National Health Laboratory Services Charlotte Maxeke Johannesburg Academic Hospital
PROJECT TITLE:	Fosfomycin Susceptibility of Uropathogens Isolated at the Charlotte Maxeke Johannesburg Hospital Microbiology Laboratory
DATE CONSIDERED:	29/09/2017
DECISION:	Approved unconditionally
CONDITIONS:	
SUPERVISOR:	Dr Trusha Nana and Dr Norma Bosman
APPROVED BY:	Professor P. Cleaton-Jones, Chairperson, HREC (Medical)
DATE OF APPROVAL:	11/12/2017

This clearance certificate is valid for 5 years from date of approval. Extension may be applied for.

DECLARATION OF INVESTIGATORS

To be completed in duplicate and **ONE COPY** returned to the Research Office Secretary on the 3rd floor, Phillip Tobias Building, Parktown, University of the Witwatersrand. I/We fully understand the conditions under which I am/we are authorised to carry out the above-mentioned research and I/we undertake to ensure compliance with these conditions. Should any departure be contemplated, from the research protocol as approved, I/we undertake to resubmit to the Committee. **I agree to submit a yearly progress report.** The date for annual re-certification will be one year after the date of convened meeting where the study was initially reviewed. In this case, the study was initially review September and will therefore be due in the month of September each year. Unreported changes to the application may invalidate the clearance given by the HREC (Medical).

Principal Investigator Signature

Date 13-12-2017

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES